

# HbS/ $\beta^{\text{del}}$ -Thalassemia Associated With High Levels of Hemoglobins A<sub>2</sub> and F in a Turkish Family

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$\beta$ -thalassemia and sickle cell disease (SCD) are common disorders in Turkey. Compound heterozygosity for these two disorders ( $\beta^{\text{S}}/\beta$ -thalassemia) is encountered frequently. In this report we present hematological and molecular data of two Turkish siblings with  $\beta^{\text{S}}/\beta^{\text{del}}$ -thalassemia caused by a 290 base pair (bp) deletion and associated with increased levels of hemoglobin A<sub>2</sub> (HbA<sub>2</sub>) and hemoglobin F (HbF). Clinical analysis of the two patients showed a mild course of the disease. Haplotypic factors involved in increasing the levels of HbF were analyzed. The two patients showed no changes from the normal sequences at the XmnI site of G $\gamma$ -globin promoter and the (AT)<sub>x</sub>T<sub>y</sub> microsatellite 5' to the  $\beta$ -globin mRNA cap site. The removal of the region between positions –125 to +78 relative to the  $\beta$ -globin gene mRNA cap site by the 290 bp deletion is thought to allow the  $\beta$ -locus control region to interact with the promoters of the  $\delta$ - and  $\gamma$ -globin genes, leading to increased HbA<sub>2</sub> and HbF levels. Am. J. Hematol. 59:83–86, 1998.

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**Key words:**  $\beta$ -thalassemia; sickle cell disease; hemoglobin A<sub>2</sub>; hemoglobin F; haplotype; Turkey

## INTRODUCTION

$\beta$ -thalassemia and sickle cell disease (SCD) are common disorders in Turkey. The overall estimated frequency of  $\beta$ -thalassemia is 2% [1]. SCD, however, is not distributed homogeneously in Turkey and is almost limited only to one group of people (Eti-Turks) living along the country's south-eastern coasts where the disease frequency varies from 0.3 to 37% [2]. In that part of the country, consanguineous marriages are commonly practiced, and since  $\beta$ -thalassemia and hemoglobin S (HbS) are relatively frequent in that region, compound heterozygosity for these two disorders ( $\beta^{\text{S}}/\beta$ -thalassemia), usually expressed in a severe type of disease, is not rare [2]. In this report, we present hematological and molecular data of two Turkish siblings with  $\beta^{\text{S}}/\beta^{\text{del}}$ -thalassemia caused by a 290 base pair (bp) deletion involving part of the 5'  $\beta$ -globin gene region and associated with increased levels of hemoglobin A<sub>2</sub> (HbA<sub>2</sub>) and hemoglobin F (HbF).

## SUBJECTS AND METHODS

### Patients

The family members, father, mother, and three siblings, from the city of Batman (Eastern Anatolia), were

first diagnosed in the Hematology Clinic of Cerrahpaşa Medical School (Istanbul, Turkey). Blood samples from all members were collected in ethylene diaminetetraacetic acid-containing tubes. Informed consent was obtained. HbS, HbF and HbA<sub>2</sub> values were determined by hemoglobin electrophoresis. Results were confirmed for HbF and HbA<sub>2</sub> by alkaline denaturation and column chromatography, respectively.

### Molecular Analysis

The presence of the 290 bp deletion was shown by polymerase chain reaction (PCR) amplification of the  $\beta$ -globin gene using primers flanking the deletion (Table I). The breakpoints of the deletion were defined by direct

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TABLE I. PCR-Based Methods of Analysis for the 290 bp Deletion and the XmnI-G $\gamma$  and (AT) $_x$ T $_y$  Polymorphisms\*

Region analyzed	Primers	PCR profile	Resulting fragment length
290 bp deletion	CD7 5'-TCCTAAGCCAGTGCCAGAAG-3' R109 5'-CCCTTCCTATGACATGAACTTAACCAT-3'	95°C, 4'; (95°C, 30"; 55°C, 30"; 72°C, 2') 30 cycles; 72°C, 5'	727 and/or 435 bp
XmnI G $\gamma$ -polymorphism	G5 5'-GAGCTACAGACAAGAAGGTG-3' G4 5'-TTTTATTCTTCATCCCTAGC-3'	94°C, 1'; (94°C, 30"; 52°C, 30"; 72°C, 1') 35 cycles; 72°C, 5'	351 bp
(AT) $_x$ T $_y$	$\beta$ B 5'-CTACCATAATTCAGCTTTGGGAT-3' $\beta$ D 5'-CCTCACCTGAGGAGTTAATT-3'	94°C, 1'; (94°C, 30"; 50°C, 30"; 72°C, 1') 35 cycles	768 bp

\*PCR, polymerase chain reaction; bp, base pairs.

TABLE II. Genotypes Hematological Data, and Hemoglobin Constitutions of the Family\*

Subject	Sex-age (years)	Relation-ship	Genotype	XmnI	(AT) $_x$ T $_y$	Hb (g/dl)	Hct (%)	MCH (g/dl)	RBC ( $10^{12}/l$ )	MCV (fl)	MCH C (pg)	Reticulo-cytes (%)	HbA $_2$ (%)	HbF (%)	HbS (%)
Ha. Y.	M-32	Father	-290 bp/N	-/nd	(AT) $_7$ T $_7$ /nd	13.2	43.4	20.2	6.54	66.4	30.4	2.6	9.0	0.8	0.0
Se. Y.	F-28	Mother	HbS/N	-/nd	(AT) $_7$ T $_7$ /nd	13.2	40.2	27.5	4.81	83.7	32.9	1.6	3.3	0.5	43.2
Ah. Y.	M-10	Son	-290 bp/HbS	-/-	(AT) $_7$ T $_7$ /(AT) $_7$ T $_7$	9.5	27.5	31	4.05	71	22	31	6.7	18.1	50.7 <sup>a</sup>
Mu. Y.	M-9	Son	N/N	nd/nd	nd/nd	12.9	39	27.8	4.63	84.2	33.0	1	3.5	0.6	0.0
Me. Y.	F-5	Daughter	-290 bp/HbS	-/-	(AT) $_7$ T $_7$ /(AT) $_7$ T $_7$	10.7	32.5	31.9	4.76	69.1	22	1.2-5.8	6.0	33.6	60.4

\*Hb, hemoglobin; nd, not determined.

<sup>a</sup>The total quantity of hemoglobin types in the proband is 75.5%; the remaining 24.5% is transfused HbA.

sequencing of the abnormal PCR product [3]. Putative cis-acting determinants modulating HbF levels within the  $\beta$ -globin gene cluster were analyzed. The -158 C-T polymorphism in the G $\gamma$ -globin gene promoter was assessed by XmnI restriction of a 351-bp amplified sequence from that region (Table I). The AT-rich region at 530 bp upstream from the  $\beta$ -globin gene was analyzed by amplifying and directly sequencing a 790 bp DNA fragment [4-6].

## RESULTS

### Clinical Phenotypes

The hematologic and hemoglobin composition data of the family members are given in Table II. The father, carrying the  $\beta$ -thalassemia deletion, had an unusually elevated HbA $_2$  (9.0%) and normal HbF levels. The mother showed a typical HbS heterozygote hematology. Two out of their three children inherited both defects from their parents, whereas one boy, Mu. Y., was healthy. One of the two affected siblings, Ah. Y., was a 7-year-old boy at the time of diagnosis (1994). From his fourth year of life, he had pains in his hand and foot joints, and occasionally the color of his urine turned dark. Physical examination of the patient showed paleness, 3.5 cm hepato- and 8 cm splenomegaly. Up to this age, he was not transfusion-dependent. These findings seemed to be in accordance with a  $\beta^S/\beta^0$ -thalassemia condition. The child Ah. Y. is currently transfused, not because of anemia, but because of recurrent pains in his extremities every two months. He is currently 11 years old, and his liver and spleen are 2.5 and 10 cm, respectively. The

same diagnosis was made in the patient's sister, Me. Y. At the time, Me. Y. was 1 year old and showed no hepatosplenomegaly and did not need transfusion. She is currently 6 years old and has a very stable and symptomless condition. Her liver is 2 cm and she has no splenomegaly. These findings mistakenly predicted the presence of a  $\beta^S/\beta^+$ -thalassemia genotype but were corrected later by the results of the molecular analysis.

### Molecular Analysis

Genotypes of all the family members were revealed by PCR and genomic sequencing (Table II). The 5'-breakpoint of the 290 bp deletion is 123-125 bp upstream to the  $\beta$ -globin gene cap site and the 3' end is at nucleotide 23-25 of IVS-I of the globin gene [7-10]. Analysis of the -158 C-T polymorphism in the  $\gamma$ -globin gene promoter showed no change from the normal sequence (Table II). The same result was obtained for the (AT) $_x$ T $_y$  microsatellite, which showed an (AT) $_7$ T $_7$  repeat, as in the normal reference sequence of Poncz et al., 1983 [11].

### DISCUSSION

Due to their relatively frequent occurrence in Turkey,  $\beta$ -thalassemia and HbS have a high chance to coexist in many Turkish patients [2]. The outcoming phenotypes are usually heterogeneous and mainly dependent on the  $\beta$ -thalassemia mutation inherited [12]. Several reports have described  $\beta^0$ -thalassemia mutations coexisting with HbS in patients from different populations. However, only four reports have recorded the association of a de-

lethal type of  $\beta$ -thalassemia (1.35 kbp or 532 bp deletions) with HbS in the same patient [13–16]. The 290 bp deletion was first observed in a Turkish patient [7] and then in several others [8–10]. In our present investigation, we describe the first occurrence of the 290 bp deletion along with HbS. Although carrying two deleterious types of mutations, our patients have mild symptoms due to their increased levels of HbF (18.1 and 33.6%) and HbA<sub>2</sub> (6.7 and 6%). Under conditions of erythropoietic stress, HbF might remain a little increased in early childhood, but the hemoglobin levels in our patients are too high for this, and their ages are already 10 and five.

Increased levels of HbF interfere with the polymerization of HbS molecules within the red cell and, therefore, ameliorate the cycle of sickling/unsickling which ultimately leads to the irreversibly sickled cell and cell death. Factors associated with the  $\beta$ -globin haplotypes play an important role in the determination of HbF levels in patients with hematopoietic stress. Four common  $\beta^{\text{S}}$ -globin haplotypes exist in the world, Benin, Cameroon, Senegal, and Arab-India. The latter two haplotypes were demonstrated to be always linked to increased levels of HbF in either  $\beta^{\text{S}}/\beta^{\text{S}}$  or  $\beta^{\text{S}}/\beta$ -thalassemia patients, no matter whether these haplotypes were homozygously or heterozygously inherited [17–19]. The increased HbF levels may have been due to such genes bearing an XmnI site at nucleotide –158 5' to the  $\gamma$ -globin gene [20]. Recent studies have shown that the (AT)<sub>9</sub>T<sub>5</sub> microsatellite sequence, found in the Arab-India haplotype [21] at –530 bp 5' to the  $\beta$ -globin gene, has an increased affinity to a negative transacting factor named BP-1 [22]. Upon binding tightly to the (AT)<sub>9</sub>T<sub>5</sub> sequence, BP-1 represses the  $\beta$ -globin expression, thus decreasing the HbS production in the case of  $\beta^{\text{S}}$ -genes and contributing to the mild course of the disease. The microsatellite occurs as (AT)<sub>7</sub>T<sub>7</sub> in all other  $\beta^{\text{S}}$ -haplotypes as well as in many  $\beta$ -thalassemia and normal haplotypes [11], and all these cases have a low binding affinity to the BP-1 protein. We tested our two  $\beta^{\text{S}}/\beta$ -thalassemia patients for the XmnI polymorphism and the AT<sub>x</sub>T<sub>y</sub> microsatellite sequence. Our results showed no changes from the normal sequence at these two positions, i.e., C at position –158 5' to the  $\gamma$ -globin gene, and (AT)<sub>7</sub>T<sub>7</sub> at –530 bp 5' to the  $\beta$ -globin gene. This result is further proof that  $\beta^{\text{S}}$ -genes in Turkey appear to be particularly specific for the Benin haplotype (96%) that is usually associated with low HbF values [23]. Since the hematology of the parents did not show any change from the normal, the dominant X-linked F-cell production (FCP) determinant is not expected to be the reason for the observed phenotype [24–27].

The majority of patients with  $\beta^{\text{S}}/\beta^0$ -thalassemia (due to point mutations) had severe disease with many clinical complications similar to those of severe  $\beta^{\text{S}}/\beta^{\text{S}}$  patients [19]. However, all  $\beta^{\text{S}}/\beta^{\text{del}}$ -thalassemia patients, reported

so far, exhibited a mild phenotype with increased levels of HbA<sub>2</sub> and HbF [13–16]. The reason for this observed phenotype is the  $\beta^{\text{del}}$ -thalassemia gene, the 290 bp deletion in the case presented here, and not the  $\beta^{\text{S}}$ -chromosome. The common outcome between these  $\beta$ -globin gene deletions is the removal of the region between positions –125 to +78 relative to the  $\beta$ -globin gene mRNA cap site. Some of the elements deleted in this region are the CAC (–90), CAAT (–70), and TATA (–30) boxes. The absence of these elements is thought to be of great functional significance in eliciting the unusually high HbA<sub>2</sub> and HbF phenotypes. Two principal hypotheses may explain the effect of the 290 bp deletion on the expression of  $\delta$ - and  $\gamma$ -globin genes:

- 1) The deletion of sequences around the  $\beta$ -globin gene decreases the distance separating the  $\delta$ - and  $\gamma$ -globin genes from the enhancer sequences located 3' to the  $\beta$ -globin gene. On a chromosomal scale, the 290 bp deletion is not expected to significantly translocate the enhancer element closer to the  $\delta$ - and  $\gamma$ -globin genes. However, it may have a relevant effect at the three-dimensional level of the chromatin structure in that chromosomal location [28].
- 2) The second and more probable hypothesis involves a competition between the fetal and the adult globin genes for the hypersensitive site in the upstream locus control region (LCR). In the absence of the 5'  $\beta$ -globin gene promoter, the LCR would interact with either the  $\delta$ - or  $\gamma$ -genes enhancing their expression [29,30].

Molecular examination of these two mechanisms will shed light to the interactions causing the increase in HbA<sub>2</sub> and HbF levels in our two patients.

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